Development of a Research Strategy for Assessing the Ecological Risk of Endocrine Disruptors

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ABSTRACT

Correlational evidence suggests that specific populations of animals have been, or currently are being, adversely affected by exposure to environmental contaminants that manifest effects through different endocrine systems. However, there currently are insufficient data to resolve the ecological risk associated with endocrine-disrupting chemicals (EDCs). In recognition of this uncertainty, the Office of Research and Development (ORD) of the U.S. Environmental Protection Agency (EPA)sponsored a workshop that focused on a variety of issues central to the development of a research strategy for assessing ecological effects of endocrine disruptors. Specifically, the workshop was intended to address topics and concepts that would contribute to a strategy designed to establish: 1) a research framework focused upon the greatest uncertainties confronting risk assessment and risk management decisions concerning ECDs, and 2) proactive coordination and communication among Federal agencies whose research missions are especially relevant for the many different facets of this issue.

During the first two days of the workshop, more than 60 international experts in the areas of risk assessment,

comparative endocrinology and environmental toxicology were involved in presentations and discussions pertaining to the potential ecological risk of EDCs. On the final two days of the workshop, a group of Federal scientists representing EPA, the U.S. Fish and Wildlife Service, the National Biological Service and the National Oceanic and Atmospheric Administration met to develop a research strategy based upon input from the larger meeting. These deliberations were structured in the context of the EPA ecological risk assessment framework, with special emphasis placed on evaluation of relevant measurement endpoints in the context of likely assessment endpoints, as well as exposure and effect characterization. The resultant research recommendations were developed conscious of the need to establish approaches to determine the relative ecological risk of EDCs to populations and communities, both from a prospective and retrospective standpoint, and to evaluate and potentially modify the current requirements for testing and evaluating chemicals and environmental samples to ensure that those exerting toxicity through specific endocrine axes will be adequately characterized.

Introduction

Correlational evidence suggests that specific populations of animals have been, or currently are being, adversely affected by exposure to environmental contaminants that manifest effects through different endocrine systems. There have been several recent reports of endocrine-mediated abnormalities in specific populations of invertebrate, fish, avian, reptilian, and mammalian species. For example, exposure to DDT has been associated with the feminization of gull embryos (1), and several research groups also have observed feminization of fish from waterbodies receiving discharges of municipal and some types of industrial effluents (2,3). Fish exposed to pulp and paper mill effluents have been reported to exhibit abnormal circulating levels of specific reproductive hormones, although population-level effects have not been observed (4). Delayed or abnormal sexual differentiation has been correlated with population declines of alligators and the presence of organochlorine pesticides (ostensibly DDT and metabolites) in lakes in Central Florida, most notably Lake Apopka (5). Imposex (simultaneous presence of both male and female reproductive organs) in different species of marine gastropods has been strongly correlated with exposure to tributyltin (TBT), and this condition

may be driving specific local populations of invertebrates to extinction (6,7). Egg mortality and terata in local populations of fish-eating birds from the Great Lakes have been well-documented, and seemingly are related to organochlorine contaminants, most notably PCBs, which may exert toxicity through one or more endocrine pathways (8).

These and other observations in fish and wildlife, as well as human epidemiology studies documenting, for example, decreases in sperm quality (9), have served as an impetus for various meetings/workshops focused on environmental "endocrine disruptors" (e.g.,10). This, in turn, has led to polarized scientific debates, both in the technical literature and the popular press, as to the potential level of concern that should be afforded these types of chemicals (e.g., 11-14). Based upon these ongoing debates, it appears that the emerging consensus is that there are insufficient data to resolve the relative ecological or human health risk associated with environmental contaminants that exert toxicity through alterations in endocrine systems.

In recognition of this uncertainty, the Office of Research and Development (ORD) of the U.S. Environmental Protection Agency (EPA) initiated a formal research program to evaluate the

potential risk of endocrine-disrupting chemicals (EDCs) to both human and ecological health. A key component in the initiation and development of this research program has been, and will continue to be, the involvement of scientists from other government agencies, academia, industry and public interest groups. In addition, ORD has brought an international perspective to the planning exercise through involvement of scientists and regulators from countries such as Canada, Britain, Denmark, Germany and Sweden. To facilitate research planning, ORD held an initial workshop entitled "Endocrine Disruptor Research Needs" in April, 1995 in Raleigh, North Carolina. More than 300 participants at that workshop developed a framework document broadly outlining research needs and issues for defining health and ecological risks of EDCs (15). One need identified at that workshop was to have smaller, more focused meetings concerning specific research areas. In response to this, a workshop entitled “Ecological Effects of Endocrine Disruptors”, was held concerning research needs and directions associated with the ecological risks of EDCs.

The workshop was held June 13-16, 1995 in Duluth, Minnesota, and was conducted in a phased manner. During the first two days, a group of approximately 60 participants from a variety of governmental agencies, academia, industry, and public interest

groups (Table 1) met to exchange information, broadly discuss limitations in existing data, and identify research needs. During the last two days of the planning exercise, government scientists from the EPA, the U.S. Fish and Wildlife Service (FWS), the National Biological Service (NBS) and the National Oceanic and Atmospheric Administration (NOAA) Marine Fisheries Service (Table 1) met to develop a specific research strategy based upon input from the larger meeting. In the following section, we describe the agenda, input and recommendations associated with the first two days of the meeting.

OVERVIEW OF THE ISSUE AND RECOMMENDATIONS: DAYS 1,2

The first day of the workshop consisted of a series of lectures and discussions relative to specific issues, systems and species in terms of endocrine disruptor effects and research. Presentations ranged from approximately 25 to 45 min in length, followed by a period for questions and discussion (Table 2). At the conclusion of each talk, the individual presenters identified various research needs germane to their particular topic. These recommendations, together with research needs identified at the April workshop at Raleigh (Table 3) served as a

basis for identifying breakout group discussion topics for the second day of the meeting.

Plenary Lectures

After a welcome from Dr. Gilman Veith (ORD, EPA), Dr. Gerald Ankley presented background information concerning the efforts of ORD in developing a research program dealing with endocrine disruptors. In his talk, the framework and recommendations from the initial April meeting were summarized, one of which was that a series of more focused workshops on research planning be conducted (15). Dr. Ankley also presented the working definition, developed at the Raleigh meeting, of an EDC: "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and regulation of developmental processes" (15).

Following the introductory presentations, Dr. Glenn Suter provided an overview of the ecological risk assessment paradigm currently used by EPA (16,17). Critical differences between human and ecological risk assessments were stressed, with special emphasis placed on the concept that ecological assessments focus

on population-, or occasionally, community-level effects, while human assessments focus on the individual (18). Dr. Suter's presentation subsequently stressed the importance of identifying measurement endpoints, which are typically at the level of the individual, that reflect assessment endpoints of concern (e.g., trends in populations and communities), noting the need for developing and validating linkages between the two types of endpoints. In addition, the importance of acknowledging and quantifying uncertainty in risk assessments was addressed, particularly in the context of identifying key research areas where the greatest uncertainty exists in predicting or interpreting the potential impacts of EDCs. Finally, Dr. Suter felt that reducing ecological effects of EDCs would in many instances ensure that human health also was protected.

Dr. David Norris then provided an overview of comparative endocrinology where he identified potentially sensitive targets of EDCs by lifestage (early development, sexual maturation, reproduction, senescence) and endocrinological axes (especially adrenal, thyroid and reproductive systems). He noted that, in general, certain aspects of several endocrine systems are remarkably well conserved across phyla pointing, for example, to reproduction (19,20). Based upon this observation, it was noted that EDCs that act through specific receptors (affecting hormone

synethsis, release and/or actions) could well be particularly amenable for extrapolation of prediction of relative risk across species and, as such, might provide a convenient model for this type of exercise.

Following the presentation by Dr. Norris, there were a series of five talks focused specifically upon processes at risk. The purpose of these presentations was to better define those lifestages and endpoints upon which research could be most productively focused, both in terms of potential ecological effects and uncertainty (i.e., current lack of knowledge). Although the presentations were made by scientists involved in specific systems/species, an effort was made to have the presentations be comparative in nature.

Dr. Peter Thomas discussed key points at which reproduction could be impacted and presented several examples from his research on the effects of cadmium, crude oil and PCBs on reproduction in fish (21-25). He also pointed out that, despite the current emphasis on steroid hormones, the neuroendocrine system should not be ignored.

Dr. Jennifer Specker then discussed the role of endocrine systems in terms of growth and development. This presentation

emphasized the role of thyroid hormones, which thus far have not received as much attention as steroid hormones in the context of EDC effects. She pointed out that the thyroid hormones are well conserved across phyla (26), and suggested that amphibians may be good models for investigations concerning the system. Dr. Specker also noted that the maternal transfer of thyroid hormones (T3/T4) via the yolk in fish, for example, might be a key stage for potential disruption (27-30).

Dr. Carl Schreck discussed the role of endocrine involvement in immunology and stress response (31-33). He felt that stress response and development of the immune system might be particularly prone to effects mediated by EDCs (34,35). Dr. Schreck also discussed the need of integrated laboratory and field studies specifically focused upon correlation of pathogen load/immune response with contaminant exposure, and presented results of a study of this type from his laboratory which evaluated salmonids exposed to a pulp and paper mill effluent.

In the following presentation, Dr. Steven McCormick discussed the role of endocrine systems in osmoregulation. Prolactin, cortisol, thyroid hormones, renin-angiotensin, catecholamines and natriuretic peptides are important in ion and water balance and are greatly altered during transitions between

fresh water and seawater (36,37). Although numerous compounds exert their toxic effects by damage to osmoregulatory mechanisms (38), there is currently no known link between endocrine disruption and biologically significant alterations in osmoregulation. However, certain life history stages such as the parr-smolt transformation of anadromous salmonids and animals in estuarine habitats may be particularly susceptible to endocrine disruption of osmoregulation.

In the final presentation on processes at risk, Dr. David Crews discussed the role of endocrine systems relative to behavior. As with osmoregulation, he felt that there currently is too little information to link chemical disruption of specific hormonal systems to biologically significant changes in behavior. Dr. Crews did present several examples, however, where subtle effects on behavior could seriously alter reproductive success (39-41).

A series of presentations then focused upon major groups of animals potentially at risk: fish, birds, reptiles, (marine) mammals and invertebrates. An obvious omission from this list was amphibians, not because of a lack of concern, but because of a current lack of specific examples of adverse effects of EDCs in this class of organisms in an environmental setting. However, it

was pointed out by several workshop participants that amphibians might be an important group of animals on which to focus, particularly in light of recent population declines of several species within this class (42).

Dr. Glen Van Der Kraak presented data from a series of studies focused upon the effects of pulp and paper mill effluents on endocrine function in fish. In those studies, it was clear that exposure to the effluents induced hepatic monooxygenase activities, altered circulating levels of sex steroids, and caused delayed sexual maturation in white sucker; however, these changes were not directly correlated with population-level impacts such as relative abundance of the fish (4,43-45) Based on those findings, Dr. Van Der Kraak noted the importance of establishing linkages between individual- and population-level effects. He also discussed the potential utility of toxicity-based fractionation approaches for identifying specific chemicals of concern in the complex pulp and paper mill effluents (e.g., 46,47).

Dr. Michael Fry discussed the significance of EDCs relative to impacts on different avian populations, with a primary emphasis on reproductive function and/or development in different piscivorous species including ospreys, cormorants, gulls and

eagles (48-51). Dr. Fry suggested that appropriate measurement endpoints for assessing the possible biological effects of EDCs in wild populations would include survival to hatch (i.e., viable eggs), skewed sex ratios, abnormal reproductive behavior and teratogenic deformities (1,8,52-56). He also indicated the need for further research on EDCs and passerine species, in part because several populations appear to be declining (42).

The effects of EDCs on reptiles was addressed by Dr. Louis Guillette who described studies conducted on Lake Apopka where there appear to be population-level impacts due to chemically-induced feminization of male alligators (5,57,58). He also indicated that similar problems may exist in other central Florida lakes, suggesting that this problem is not an isolated phenomenon in Lake Apopka. Dr. Guillette noted that a key to research programs evaluating the potential ecological effects of EDCs is the collection and evaluation of high-quality population monitoring data; he pointed out that most effects demonstrated to date have originated from this type of information. He also suggested that certain reptilian species could serve as useful and sensitive laboratory models for screening EDCs (e.g., sexual differentiation in turtles; 59).

Dr. Mats Olsson presented the results of studies conducted on seals from the Baltic Sea (60). In the 1960s and 70s, marked declines in populations of Grey Seals were noted, and a series of integrated field and laboratory studies were conducted to define the etiology of the declines (61-64). These studies were unique in terms of their scope, but more importantly, they represent species that are very difficult to study, and because of their position at the peak of aquatic food webs, may represent an exceptionally vulnerable group of organisms. Dr. Olsson and coworker's have suggested that declines in the seal populations may have been related to an adrenal cortex disorder caused by PCB and DDE methylsulfones (60,65,66). In concluding his presentation, Dr. Olsson also stressed the importance both of multidisciplinary approaches and the need for an international perspective in EDC research.

The influence of EDCs on invertebrate species was addressed by Dr. Gerald LeBlanc, who noted that greater than 90% of all animal species potentially affected by these types of chemicals are invertebrates. He pointed out that abundant information on certain sexual and developmental hormones in different insect species has been collected in conjunction with the development of pesticides (67). As had several previous speakers, Dr. LeBlanc commented on the degree of conservation of endocrine system

structure and function across phyla. He also presented specific examples, both from laboratory (68,69) and field (70) investigations of adverse effects related to alterations in endocrine function in invertebrates, with the most prominent example being the observation of imposex in different marine gastropods exposed to TBT (6,7,71).

The final two presentations, by Dr. Glen Fox and Dr. Steven Bradbury, addressed issues more generic to the evaluation of existing or potential ecological effects of EDCs. Dr. Fox described a paradigm for developing plausible cause and effect relationships in retrospective risk assessments, commonly termed “ecoepidemiology”. In this analysis, causal relationships are based upon a "weight of the evidence" approach that includes consideration of: 1) time order, 2) strength of the association, 3) specificity of the association, 4) consistency of the association, 5) coherence of the association, 6) probability, and 7) predictive performance (72). This approach has been successfully applied to a number of contaminant-related ecological impacts, most notably evaluation of the effects of PCBs (certain of which are related to endocrine function) on various piscivorous birds in the Great Lakes (73,74).

Dr. Bradbury presented an overview of the use of structure-activity relationship (SARs), which are models that relate chemical structures and properties to biological activity, in ecological risk assessments (75), and their potential use for evaluating potential EDCs. For example, SARs could be used to identify analogues of hormones and/or predict binding efficiency and levels of agonistic or antagonistic activity to support screening-level risk assessments. In addition, these models can be used to help identify those chemicals that may require in-depth toxicological study to support more extensive risk assessments. SARs have been used in the pharmaceutical and agrochemical discovery area and there also have been reports on the use of these models for screening industrial chemicals for "hormonal activity" (e.g.,76). The discussion then centered on a series of examples that illustrated the need to establish well-defined endpoints in SAR research and how modeling uncertainty can be reduced through a mechanistically-based appreciation of ligand/receptor interactions and associated biological activity (77-79).

Breakout Group Activities/Recommendations

At the conclusion of the first day, workgroup chairs and rapporteurs met to finalize specific discussion topics for the second day. Three breakout groups were established to address topics related to integration/implementation concerns, field considerations, and laboratory issues. Final discussion topics were based on: 1) general research needs identified at the April workshop in Raleigh (Table 3), 2) research suggestions by the presenters on Day 1, and 3) needs/concerns specific to the role of EPA/ORD.

Integration and Implementation

Research Issues

There was a strong opinion from this workgroup that several general issues needed to be addressed in conjunction with the development of specific recommendations. These can be summarized in four points.

1) It is extremely difficult to plan a meaningful research strategy for an issue as broad as the endocrine disruptor issue. The workgroup believed that the development of useful research plans required focusing by "writing down the goals and objectives

of the federal research program ” as part of the planning process.

2) The term endocrine disruptor, or more correctly neuroendocrine disruptor, is not very well defined. It might be helpful if a more precise definition could be obtained. The definition used at the Raleigh meeting (15) may be too broad because, by definition, nearly any toxicant would be defined as an endocrine disruptor. A better definition would be one which does not include all toxicants (by virtue of secondary homeostatic endocrine mediated responses), but rather, focuses on those likely to causes adverse effects on individual organisms through primary effects on endocrine systems that could lead to population- and community-level impacts.

3) All ecotoxicological research is or should be done in context. That is, there should be an over-arching research strategy that establishes guiding principles that organize the work. Specifically, planned research should integrate the following two axioms of Warren (80):

The significance of observations at one level of biological organization is obtained by looking at higher levels of organization.

The mechanistic explanation of observations is obtained by looking at lower levels of organization.

Furthermore, planned research activity should incorporate a focus upon integration and interdisciplinary efforts in addressing the problem.

4) Prudence dictates that efficient research strategies rarely rise de novo but rather are built from an existing framework. The process for developing the endocrine disruptor research strategy should be to:

Specifically re-evaluate the existing testing models, frameworks, and endpoints with respect to how well they address the issues posed by endocrine disruptors. Then, modify the models, frameworks, and endpoints as necessary and appropriate to incorporate the required mechanisms. Special recognition of the importance of dose-response as it relates to endocrine disruptive effects should be emphasized.

Research Strategies

It was the consensus of the workgroup that the following endocrine functions should be considered the most significant in environmental effects research:

- Reproduction
- Growth/Development
- Immunocompetence

Further, it was felt that from the standpoint of ecological risk assessment, reproduction and growth/development research should have a higher priority than work related to immunological effects of EDCs.

The workgroup made specific recommendations in the following areas.

1) Animal models: When selecting model vertebrates for research activities, careful consideration should be placed on how well the model selected represents the ecologically-important groups that are at highest risk of adverse impact. There are some vertebrate groups, such as teleosts, that are well-studied and have representative species that can be easily evaluated in the laboratory. Conversely, other ecologically important groups,

such as anuran amphibians, lack adequate laboratory models. For example, while *Xenopus* may exhibit some endocrine functions common to all amphibians, it might not exhibit the critical endocrine functions of some of the endangered anurans of North America. Other examples of critical vertebrate classes that lack representative laboratory models include turtles and other reptiles, passerine birds, non-teleost fishes (including sturgeons) and non-rodent mammals.

Another issue considered important to the selection of animal models was basic research into invertebrate endocrinology, particularly for non-arthropods. This is especially important because the state of our knowledge in this area is weak and, in some ecosystems, invertebrates are keystone species.

2) Modeling issues: Several issues regarding the development of conceptual models that incorporate endocrine disruption mechanisms were discussed:

a) Lab to field research should be linked by developing mechanism-based dose response models. Furthermore, exposure levels observed in the field should be used as a basis for identifying realistic dose ranges in laboratory experiments.

b) Researchers should proceed from first order models to higher order models. It is critical to analyze the range of uncertainty present in the model with the expected uncertainty in the independent parameters to see if the model can be useful.

c) There is a need for better individual-based population models to allow prediction of potential field effects from laboratory results. Coupled to this is a need for better models that include parameters describing demographics as well as likely exposure distributions.

d) Better toxicokinetic models would allow more accurate prediction of tissue and cellular dose during pulsed exposures to chemicals with various physico-chemical characteristics, especially at critical and sensitive early life-stages. Similarly, toxicodynamic models are especially needed to understand the role of metabolism in the activation and/or elimination of potential endocrine disruptors. Receptor-based toxicodynamic models are poorly developed.

e) SAR models that describe specific and non-specific binding of endogenous and exogenous ligands to carrier

proteins and to receptors would allow identification of potential EDCs for further empirical testing.

3) Mixtures: There are two critical issues to address when considering EDCs in the context of real-world scenarios where organisms are exposed to multiple chemical stressors during different lifestages. First, the organizational effect of a disruption during embryonic development might not be observed or expressed until much later in the animals life, perhaps not until an activational hormone stimulus is received (81). Secondly, unlike most mixtures (where additivity of toxic equivalence is generally considered to conservatively predict the total mixture toxicity), the potential for synergism may be high for endocrine disruption mechanisms.

Field

Research Issues

Each workgroup member was asked to provide a single topic for consideration by the full workgroup, and subsequent discussion expanded these topic areas. Research issues and strategies were developed with the following assessment question in mind: "What is the actual risk of endocrine disruption (as

opposed to other stressors) to any given ecosystem?". The important research issues/concerns identified were as follows:

1) Adequacy of existing monitoring frameworks: The group felt that it was critical to be able to document the extent and magnitude of both exposure to and effects of EDCs in the field. Case studies to date have often been identified through "serendipity", and it is not known how representative these examples are of a larger-scale problem. In this respect, it is necessary to have a more systematic approach to identifying problems suspected due to EDC exposures.

There are existing monitoring programs collecting data that could be used to help in problem formulations for risk assessments, or to support exposure or effect characterizations in retrospective risk assessments. Potential examples in the U.S. include the Environmental Monitoring and Assessment Program (EMAP) of the EPA, the National Status and Trends Program (BEST) of the NBS, the National Water Quality Assessment Program (NAWQA) administered by the U.S. Geological Survey, and a variety of state and joint international monitoring programs. However, participants stressed the need for: a) better uses and communication of existing exposure and effects data, b) specimen banking, and c) possible revisions to existing measurement

endpoints to ensure that they are specific to, or diagnostic of, EDC effects (see below). Finally, there is a need to identify suitable historical control/reference data for assessment of effects.

2) Adequacy of available assessment and measurement endpoints: The group felt that the choice of assessment endpoints (i.e., populations at risk) was an important issue, although often outside the purview of scientists. Although either endangered species or commercially-valuable species could be chosen as assessment endpoints, either of these approaches can lead to problems in the identification of suitable measurement endpoints. There is a lack of non-invasive methods for measuring both exposure and effects endpoints for endangered species. Assessment of EDC effects on commercially-valuable species is complicated by effects of commercial harvesting.

There is a need for a broader suite of ecologically-relevant laboratory test endpoints (e.g., metamorphosis, par/smolt transformations) to facilitate lab-to-field extrapolations. Population-level endpoints that are specific to EDC effects are desirable. Better predictive biomarkers are also needed as indicators of exposure.

3) Need for field studies: Participants felt that it was necessary to be able to confirm cause-and-effect relationships in the field. In this respect, there is a need for better coordination between laboratory and field studies. Field studies also are necessary to establish the ecological relevance of effects at higher levels of biological organization. For example, case studies to date have not established whether there are community or ecosystem-level effects of exposure to EDCs, but have tended to focus on the organismal level for one taxon or trophic level. Studies generally have not been performed across different taxonomic groups at comparable sites.

Research Strategies

1) Monitoring frameworks: It is not necessary or desirable to establish a new monitoring program to detect effects of EDCs in the field. However, a strategic approach for using or modifying existing monitoring programs to assess current and historical effects of EDCs should be developed. This should include: a) development of a database of chemicals known to cause reproductive and developmental effects cross-referenced with chemicals known or believed to have specific relationships to endocrine function, b) identification of loading estimates for

determining exposure to potential EDCs, c) identification of appropriate reference sites, and d) consideration of potential sensitivities of specific populations given loading estimates, geographic distribution, and life history traits influencing susceptibility to EDCs. The comparability of measurements made by different monitoring programs needs to be established. The data should identify which populations are most susceptible and distinguish normal and abnormal population structures.

2) Assessment and measurement endpoints: Appropriate sentinel species for monitoring need to be identified. Considerations for their selection should include: a) representation of different life history strategies (both simple and complex), b) species vagility with respect to exposure distribution, c) representation of multiple taxonomic and trophic levels, d) manipulability of species in laboratory tests, e) availability of baseline information, and f) the degree of distribution (i.e., widespread or local) of a species. Surrogate (nonendangered, noncommercial) species for study need to be identified for which effects can be related to endangered or commercially important species.

Both EDC-specific measurement endpoints as indicators of exposure and more general measurement endpoints to establish the ecological relevance of effects should be identified. Biomarkers

must be calibrated to adverse individual- and population-level effects. Field evaluations of these markers should establish which are most predictive of population-level effects (i.e., which are most useful for establishing cause and effect relationships). This necessitates the evaluation of "normal" values and the uncertainty associated with their measurement.

3) Field studies: Focus should be directed to specific sites which are known to be affected by EDCs. Integrated laboratory and field studies should be conducted based on these case studies, but overall an ecosystem approach should be adopted; multiple phylogenetic groups and trophic levels should be studied at a given site. Retrospective assessments should follow Hill's criteria for establishing causes-and-effect relationships (82).

4) Extrapolation through modeling: It is necessary to develop predictive, integrated ecosystem models that effectively utilize SAR, toxicokinetics, bioenergetics, environmental chemistry, and population ecology models.

5) Improved communications: Communication among researchers in this area should be facilitated. Potential mechanisms include use of a newsletter or INTERNET. Monitoring data should also be

centralized, making it available on STORET, “bulletin boards”, or INTERNET.

Laboratory

Research Issues

This workgroup attempted to identify three to five of the most important research needs that can be addressed at the laboratory level for reducing uncertainty in assessing the risk of EDCs to ecosystem health. Given that the charge of the EPA/ORD endocrine disruptor workshop in Raleigh in April 1995 was to start to focus on research needs, the laboratory breakout group began its exercise using research priorities identified at the Raleigh meeting (15), as an organizing template to avoid redundancy and help identify additional areas necessary for ecological research. These research needs were:

1) Identify for the organ system and tissue considered to be at risk, the measurement endpoints to characterize risk (screening protocols in vitro, in vivo)
2) Understanding of cellular and molecular mechanisms (including non-receptor mechanisms for EDCs)
3) Sensitive, inexpensive and widely-available analytical tools

4) Ontogeny of receptor-based systems and role in regulating development
5) Identify and characterize critical windows of susceptibility across species
6) Development of biomarkers of exposure and effects of EDCs
7) Development of biomarkers for latent effects
8) Information on normal population variation, regional and seasonal effects
9) Coordinated research on exposed humans, wildlife and sentinel species
10) Target organ dosimetry for comparison with ligand binding affinities
11) Development and validation of apical methods to detect EDCs
12) Perinatal/multigenerational exposure toxicity studies for cancer and non-cancer effects
13) Laboratory-field hypothesis based studies and improved information exchange
14) Examination of correlation of effects between wildlife and human health models
15) Multi-disciplinary studies on effects of endocrine disruption
16) Improvements in study design (dose selection, endpoints, endpoint linkages)

17) Toxicokinetic and toxicodynamic studies of environmentally relevant chemicals
18) Quantitative dose response models based upon receptor theory/biochemical interactions

Several areas of endocrine-disruptor-driven research in addition to those listed in the template were added by the workgroup:

19) Bioaccumulation/biomagnification and flow of EDCs through and across trophic levels
20) Correlation between chemical concentrations and ecosystem processes that can be modeled in a lab setting
21) Population genetics - is there selective toxicity?
22) What are the driving issues? Reproduction, development, immune suppression, growth?
23) What are baseline hormone levels in individuals? What is normal endocrine status?

Guidance on research gaps given by individual speakers from the first day of the workshop tended to be specifically focused on their respective areas. However, Dr. Van Der Kraak's listing of broad "research gaps" for endocrine disruptors was given in the breakout group:

- There is a need to improve our understanding of the significance of subtle changes in endocrine performance. For example, what are the consequences of vitellogenin induction or a statistically-significant alteration in steroid biosynthesis? How do these translate to whole animal or population-level responses?

- The endocrine system involves complex homeostatic regulatory mechanisms with the result that there is a need to critically evaluate the predictiveness of *in vitro* assays. Improved understanding of the mechanism of action of chemicals will help direct the development of whole animal testing/ *in vivo* assay methods. This would help address issues such as timing of exposure, species sensitivity and interaction with different chemicals.

- There has been such a focus on chemicals with estrogenic/antiestrogenic activity that other hormones and regulatory mechanisms (e.g., immune system, vitamin metabolism) have been largely ignored.

Research Strategies

The following issues were gleaned and ranked from the original group (those with the same number were assigned equal ranks):

1) Identify for the organ system and tissue considered to be at risk, the measurement endpoints to characterize that risk (screening protocols *in vitro*, *in vivo*)
2) Understanding of cellular and molecular mechanisms (including non-receptor mechanisms for EDCs)
3) Ontogeny of receptor-based systems and role in regulating development
3) Identify and characterize critical windows of susceptibility across species
3) Development of biomarkers for latent effects
4) Sensitive, inexpensive and widely-available analytical tools
4) Development of biomarkers of exposure and effects of EDCs

Given that issues 1 and 2 could be grouped - there is a continuum of biological organization from the organ system to the tissue level to the cellular and molecular level - the remainder of the discussion revolved around identifying a unifying theme for an approach to studying chemically-induced disruption of any endocrine system. The concept of critical windows of susceptibility to endocrine disruption brought about statements

relating to the fact that most organ systems are at their highest risk when differentiating - and not only during embryonic differentiation. Critical processes (observable by functional assays) for these systems need to be identified for different developmental stages for a variety of organisms. This was refined to the need to: 1) identify critical periods during development, 2) examine the development of systems that are controlled by the endocrine system, and 3) then examine the systems when they are functioning in adulthood. This discussion set the stage for the synthesis of an EDC laboratory research model depicted in Figure 1.

Figure 1 incorporates the following synthetic elements:

- An approach for prospective risk assessment of EDCs could be modeled after the mammalian teratogen studies sometimes used by the pharmaceutical industry. Initially, standard short-term teratogenicity protocols are used and, if warranted, followed by multigenerational studies. As needed, additional endpoints appropriate for endocrine alterations can be incorporated in design of the studies. The historical equivalent in testing associated with the U.S. Food and Drug Administration are the Segment 1, 2, and 3 tests which examine exposures to the male and female followed by mating studies - exposure of both sexes and

offspring - that examine neonatal growth and behavior. These protocols could be adapted to other species such as fish, birds, mammals, amphibians, reptiles, and invertebrates.

- This framework initially focuses on screening systems followed by more extensive (and expensive) tests. There is a need for apical tests - short term, inexpensive, *in vivo* or *in vitro* assays - to screen large numbers of chemicals. These could come from available *in vitro* tests (e.g., receptor-dependent and independent endpoints, such as carrier proteins, induction of proteins such as vitellogenin, inhibition or induction of enzymes involved in steroid metabolism, enzymes involved in neurotransmitter synthesis or degradation, etc.) and *in vivo* tests (e.g., uterine weight, male accessory sex organ weight, thymic involution, vitellogenin induction).

- Biologically-based structure-activity (BBSA) models and evidence from field studies would prioritize the screening process. Historical data from retrospective studies could be used to determine what type of *in vitro*/*in vivo* screening results are the most predictive. This would help with exposure to multiple stressors, in addition to EDCs.

- Chemicals eliciting a positive response in the screen would then be examined during critical periods in the adult life cycle or in embryo/larval tests. Mechanism of action and site of action studies would follow to support the development of biomarkers and subsequently augment techniques used in screening.

In general, the model represents a conceptual framework, not necessarily a linear logic for research planning. While the organizing principles of the model are valid, research at all levels should proceed simultaneously with the ultimate goal of establishing predictive toxicology models. Within this framework, there is a need for feedback elements or what was referred to as "hypothesis modification" elements. For example, research on mechanism of action and biomarker development would enhance further EDC screens, while refined BBSA models and retrospective analysis would refine the prioritization of chemicals.

For this process, and for EDC studies in general, there was a call for a better interaction between developmental biologists studying model systems such as zebrafish, *Drosophila, Xenopus,*

and mice, and investigators studying EDCs in different classes of vertebrates and invertebrates.

DEVELOPMENT OF A RESEARCH FRAMEWORK: DAYS 3, 4

Objectives

The purpose of the final portion of the meeting was to use the input and recommendations generated during the first two days to formulate a focused research framework for defining the ecological risk associated with EDCs.

The broad objectives of this research program are framed in the risk assessment paradigm (16), and designed to address both retrospective and prospective assessments. First, it is necessary to identify the relative ecological risk of endocrine disruptors compared to other stressors on populations/communities. There was a desire, voiced both during the first two days of the workshop, and in the latter portions of the planning exercise, that research be design in such a manner that a clearer demonstration can be made that EDCs present levels of risk to populations comparable, for example, to stress due to other chemicals and/or alterations in habitat. Otherwise, research efforts might be targeted upon an issue of minimal

concern compared to other environmental stressors. As a corollary to this, the question also was posed “Are EDCs exerting effects only in relatively small populations with large exposures, or is endocrine disruption a wide-spread phenomenon?”

The second objective of this research framework is to make recommendations for developing or modifying the requirements for testing and evaluating chemicals or environmental samples so as to ensure that those exerting toxicity through specific endocrine axes will be adequately characterized. There appeared to be a consensus, both at the Raleigh and Duluth workshops, that most existing test methods likely are inappropriate for detecting EDCs because of one or more of the following factors: lack of exposure during key developmental stages, uniquely-sensitive species are not included, and/or relevant (e.g., latent) endpoints are not evaluated. It was noted, however, that this second objective, in many instances, might be accommodated with only minor modifications to existing test protocols.

Target Audience

Although this exercise was initiated specifically to explore research priorities for EPA/ORD, the concepts arising from the

workshop also are intended to serve a number of other purposes of significance to a broader audience. Specifically, the workshop was intended to address topics and concepts that would contribute to a strategy designed to establish: 1) a research framework focused upon the greatest uncertainties confronting risk assessment and risk management decisions concerning ECDs, and 2) proactive coordination and communication among Federal agencies whose research missions are especially relevant for the many different facets of this issue.

Participants and Approach

Participants in the final portion of the workshop were all Federal scientists, including representatives from the FWS, NBS, NOAA, and EPA (Table 1). EPA representation included participants from the Program Offices, as well as from human health and ecological divisions within the National Health and Environmental Effects Research Laboratory, and the Aquatic Research Division of the National Exposure Research Laboratory. The participants in this portion of the workshop also provided roughly equal representation from the three breakout groups that had convened during the second day of the meeting.

Following initial discussions about processes and species at risk, two breakout groups were formed to discuss and develop outlines identifying and prioritizing research needs and suggesting approaches to address these needs. One workgroup was charged with addressing the issue from the standpoint of retrospective risk assessments, while the other breakout group focused upon discussions from the perspective of prospective risk assessments. The charge to each breakout group was similar: to identify assessment endpoints that may be affected by EDCs, identify existing measurement endpoints for assessing the effects of EDCs, describe uncertainties associated with these measurement endpoints (particularly with respect to extrapolations to assessment endpoints), and indicate measurement endpoints and techniques that are needed, but currently unavailable. In addition, the two groups were asked to suggest generic research strategies for reducing uncertainty associated with existing measurement endpoints and the development of new measurement endpoints.

On the last day of the meeting, the two breakout groups reconvened and presented the outcomes of their deliberations in a final plenary session. These discussions formed the basis of the section entitled **DISCUSSION SYNTHESIS/RECOMMENDATIONS.**

Processes and Species at Risk

With respect to processes at risk, the consensus at the Duluth workshop was similar to that of the Raleigh workshop (15). Reproduction and development were considered the key endpoints of concern. An additional endpoint of potential significance for ecological risk assessments is immunocompetence; however, this has received little attention to date. The emphasis on reproduction as an endpoint is partially driven by the fact that, in ecological risk assessments, impacts typically are of concern at the population level. Other types of endpoints, such as histological abnormalities, which like reproduction, also are monitored at the level of the individual, may not be key in driving population dynamics. For example, certain fish-eating colonial waterbirds in the Great Lakes exhibit elevated incidences of terata, ostensibly associated with exposure to contaminants, yet their populations are not declining and are, in fact, increasing in many instances. It should be noted that if, for example, overall reproductive success is taken to be an "integrated" measure of the potential effects of EDCs on animal populations, by default this often would incorporate key processes related to development, immunocompetence, osmoregulation and behavior.

With regard to species at risk, it was felt that no particular class of organisms could (or should) receive greater attention at the exclusion of others. The limited data available from the laboratory and field suggest that, depending upon exposure history, sensitive species may include animals ranging from invertebrates to mammals. What was pointed out, however, is that there are several classes/families of organisms that have received too little attention to assess their potential susceptibility to EDCs. Prominent examples include non-arthropod invertebrates, amphibians, passerine birds, non-teleost fishes, and some terrestrial mammals, in particular those taxa where local or global populations are experiencing significant declines (42). Because of the current difficulty in a priori identification of sensitive species, it is essential to understand comparative endocrinology as it relates to EDCs.

DISCUSSION SYNTHESIS/RECOMMENDATIONS

The following summary represents a synthesis of the discussions and recommendations of the two breakout groups. These discussions are summarized within the context of effect and exposure characterization, with an emphasis on relationships

between assessment and measurement endpoints. Following these topics is a summary of research recommendations and strategies.

Effect Characterization: Endpoint Assessment

Assessment Endpoints

As a precursor to addressing the measurement endpoints relevant to evaluating the effects of EDCs, both groups discussed typical assessment endpoints for ecological risk assessments. It is not uncommon for endpoints, seemingly focused upon ecological effects, to actually be more relevant to human health concerns. For example, the presence of gross deformities in wildlife may, in some instances, be more relevant to human health concerns (i.e., using the wildlife as sentinels) than to population-level impacts in the affected species. Thus, while this may be a valid endpoint, from an ecological perspective it is important that these types of observations be relatable to discernable population- and community-level effects.

Even in this more specific context, assessment endpoints are relatively difficult to link with appropriate measurement endpoints. For example, the desire to maintain "sustainable" commercial fisheries or populations of recreational waterfowl

species certainly is a valid assessment endpoint, but these types of goals can be sufficiently vague (or complex) that it is hard to readily identify appropriate measurement endpoints.

In other instances, assessment endpoints may be to maintain viable populations of specific (often endangered) groups of socially-valuable organisms (e.g., bald eagle, Florida panther) and/or "keystone" species (e.g., lake trout, timber wolf). Keystone species may or may not be top predators or socially-valued organisms themselves, but contribute a unique trophic link or ecological role within an ecosystem such that their removal produces a cascade of significant indirect effects on other components of an ecosystem. Even in these instances, where the assessment endpoint is relatively straight-forward and quantifiable (e.g., via population census), identification of more diagnostic or predictive measurement endpoints can be problematic because a mechanistic understanding of factors controlling populations is lacking for the species of concern. For example, in Lake Ontario it is unclear whether appropriate measurement endpoints for monitoring the status of the lake trout population should be related to toxicological indices (e.g., survival of fry to swim-up), or some measure of habitat suitability (e.g., dissolved oxygen), or both.

The above discussion is, of course, not specific to evaluating the potential ecological effects of EDCs. It can be extremely difficult to identify measurement endpoints that will be useful in either retrospective or prospective risk assessments, irrespective of what stressor is responsible for population- or community-level effects. The relationship of measurement to assessment endpoints is a major issue and one which needs to be addressed in ecological risk assessment (16).

Measurement Endpoints

Existing measurement endpoints for detecting and monitoring the ecological effects of EDCs are presented based upon biological levels of organization: 1) ecosystems/communities, 2) populations, 3) individuals, and 4) suborganismal systems. In addition, the use of SAR models, which may incorporate mechanistic or empirical data at multiple levels of biological organization, are addressed. It should be noted that measurement endpoints, at any level of biological organization, are of limited value in the absence of a synoptic evaluation of appropriate exposure analysis for contaminants of concern, which is an issue that also is discussed below.

Ecosystems/Communities

Common ecosystem- and community-level measurement endpoints include structural determinants such as species assemblages and diversity (generally expressed in terms of various indices), the presence/absence of specific keystone and/or sensitive "sentinel" species, and functional measurements such as productivity and energy flow (Table 4). Although EDCs could affect one or more of these endpoints, it was felt that most would not be particularly specific to responses mediated through endocrine systems. Past research has not been designed to demonstrate a link between community structure or ecosystem function and the effects of EDCs on populations of organisms; thus, it is not known whether indicators at this level of biological organization can be developed that are diagnostic of EDC effects. However, during the first two days of the workshop, speakers discussed life history or developmental strategies that might make some species more susceptible to EDCs than others. Based upon this, it may be possible to develop a “signature” of EDC effects at the community or ecosystem level using an index related to the presence/absence of susceptible species or guilds of species. For example, fish species have already been classified based on life history strategies (83,84). In any case, even if diagnostic measurement

endpoints related to EDC effects cannot be identified at the community or ecosystem level, measurements made at these levels are critical to demonstrating the biological significance of effects at the population level.

Populations

At the population level, measurement endpoints include the number of organisms for a given species (census data), age structure, size/age relationships, sex ratios and recruitment (Table 4). The specific processes that might be assessed to yield insights about recruitment include fecundity, hatchability, and development. Age structure or size/age relationships may be indicative of past disruptions in reproduction or development and, coupled with evidence of EDC exposure history, may be useful indicators in the ecoepidemiological approach to retrospective assessments. However, as with existing measurement endpoints associated with ecosystems/communities, most endpoints at the population level are not very specific for effects that may be due to EDCs. A possible exception could be sex ratios; however, it is critical that for any given species (in a given environment), "normal" sex ratios be documented. Also, the various processes that dictate recruitment could be indicative of

disruptions in endocrine function; however, these endpoints typically are assessed at the level of the individual, with inferences subsequently made at the population level. Although many of the measurement endpoints made at the population level are not specific for effects associated with EDCs, these types of endpoints nonetheless are critical to integrated approaches either in terms of retrospective or prospective risk assessments. Further, population-level determinations are particularly important in assessing/demonstrating linkages between measurement and assessment endpoints.

Individuals

The majority of existing measurement endpoints that would be useful for assessing the effects of EDCs on reproduction, development and immunocompetence are at the organismal (individual) level (Table 4). Measurements at the organismal level range from those which integrate multiple effects (e.g., condition factors such as the gonadal-somatic index) to those which are more specific or functional (e.g., circulating levels of hormones). They also range from those that are relatively easy to interpret in terms of potential population-level effects (e.g., fecundity) to measurements, such as biomarkers (e.g.,

vitellogenin in male fish), that are not readily interpretable in terms of population impacts. Ironically, the endpoints that are closest to targeting functional effects of EDCs (e.g., circulating hormone levels, vitellogenin) are those that are least useful in terms of predicting effects in populations, as opposed to more integrative measurement endpoints associated with reproduction. Thus, there is a clear need for the development of mechanistically-based linkages both between integrative and functional organismal-level endpoints, and the prediction of population-level effects. Moreover, in this context, it is necessary to have a coherent understanding at the level of the individual of what is normal in terms of maintaining viable populations.

Measurement endpoints at the level of the individual can be made either with field-collected organisms, or with organisms exposed to single chemicals or environmental mixtures of chemicals (e.g., effluents, sediments) in the laboratory. Thus, the types of endpoints indicated in Table 4 are important to both retrospective and prospective risk assessments. Again, it should be stressed that linkage of responses, using measurement endpoints such as those described above, at the organismal level to those in populations, and perhaps communities, are critical to

integrated retrospective assessments, as well as credible prospective assessments.

With respect to the endpoints listed in Table 4, it should be noted that the majority have been examined/validated only in a limited number of species. For example, although rodent models are reasonably well developed, the extrapolation of these models to mammalian wildlife is, in general, difficult due to a lack of knowledge concerning normal endocrinology (or at least acceptable in terms of maintaining viable populations), and uncertainties related to species-specific variations in sensitivity (e.g., mink appear to be far more sensitive to PCBs than most other mammals). Similar situations exist with respect to other classes of animals: existing avian models based on the chicken, quail or mallard may not be directly relatable to raptors or passerine birds; teleost fish models, which are reasonably well developed, may not be applicable to cartilaginous fish; endocrine systems that have been characterized in arthropod invertebrates have not been compared structurally or functionally to systems in non-arthropods, etc. However, due to the relatively high degree of conservation of endocrine function among species, it should be possible to address differences that exist in a systematic manner. Thus, although these various measurement endpoints have not been evaluated in all animals of concern, the basic tools

should be broadly useful. What is required is application, adaptation and validation of the assays to different species.

An issue related to among-species extrapolations is the use of "surrogate" species in the laboratory to predict population-level responses in another (hopefully closely-related) species in the field. The uncertainty associated with this extrapolation depends not only upon the species selected, but the endpoints evaluated, and may be small (e.g., brook trout reproduction versus lake trout reproduction) or large (e.g., fathead minnow survival versus possible reproductive effects in multiple species of fish). These uncertainties exist for toxicity extrapolations for any class of chemicals, but in the case of EDCs, it might be possible to utilize among-species commonalities in endocrine systems to systematically reduce, or at least effectively quantify, certain sources of extrapolation uncertainty.

Suborganismal Systems

A number of subcellular and/or <u>in vitro</u> systems have been proposed for assessing the presence/potency of different classes of EDCs (Table 4). These systems range from receptor binding assays to measurement of functional responses (e.g., protein

induction) in normal or genetically altered cells. To date, many of these assays have focused upon steroid hormones, in particular estrogen (e.g.,85); however, viable systems theoretically could be developed for virtually any endocrine function of concern. Moreover, these types of systems should be very useful in terms both of prospective risk assessments (e.g., product screening) and retrospective analyses (e.g., to examine the "activity" of complex mixtures; (e.g., 86).

The concept of *in vitro* tests as screening tools is intuitively appealing because of cost and timeliness. However, for any system to be truly useful, it would have to be relatively sensitive and conservative (i.e., low percentage of false negatives compared to false positives), but at the same time discriminatory (i.e., able to conclusively eliminate inactive chemicals). Further, there should be a mechanistic understanding of the linkage between responses in subcellular/ *in vitro* systems and adverse effects at the level of the organism. To fulfill these various criteria, proposed subcellular and/or *in vitro* systems need to undergo more characterization and validation relative to whole organism responses. At this point, there are no assays sufficient to fully characterize the potential effects of different classes of EDCs. In fact, it might be unrealistic to expect that subcellular/ *in vitro* screening tools could serve

as more than a complement to whole-organism testing, and then perhaps only as a tool to eliminate clearly inactive substances from further consideration.

Additional tools for screening different classes of EDCs are SAR models. These models might utilize endpoints ranging from receptor binding to actual toxicity in the organism. In fact, recent efforts have resulted in promising initial models suitable for screening chemicals that bind to the estrogen or androgen receptors (87-89). However, these models require further refinement, both in terms of computational chemistry and calibration to *in vivo* toxicity. In a manner analogous to the use of subcellular/ *in vitro* screens for EDCs, SAR models would be best applied as part of a tiered testing framework that incorporates whole-organism testing as a "ground truth" evaluation of potentially active chemicals.

Exposure Characterization: Identification of Chemicals of Concern

A significant challenge in determining the extent to which EDCs may be impacting the environment is related to exposure assessment of sensitive populations to specific chemicals. An important confounding and practical aspect of this issue can

arise for sensitive populations/species because there may be no individuals present to manifest the types of effects that might alert investigators to the possibility that EDCs may be present. Thus, it is possible that there are pervasive effects of which we are not aware. In lieu of using observations of individuals or populations as indicators of effects, it becomes necessary to rely on chemical monitoring programs to help identify potential hot spots or wide-spread occurrence of specific chemicals that may cause endocrine disruption. Unfortunately, there are two problems that arise with this approach. First, support for extensive monitoring programs in the U.S. (and elsewhere) is rapidly decreasing. Second, those programs that do exist likely are not monitoring chemicals of particular concern, in large part, because there is no widely accepted "laundry list" of potential EDCs. To address this shortcoming in the near term, it may be possible to coordinate with existing monitoring programs to include routine analysis of those chemicals (e.g., certain organochlorines, alkylphenols, TBT) for which there is at least correlative evidence of potential impact. In the short term, it also should be possible to identify chemicals of potential concern through the evaluation of use patterns (e.g., production volume) and basic physico-chemical attributes (e.g., degradation rates), in conjunction with existing SAR models for the

prediction of binding affinity for specific receptors, transcriptional activity, etc.

In the longer term, as methods for defining the effects of EDCs become more refined, they can be used either in retrospective or prospective risk assessments to help identify specific chemicals of concern. Adaptations of toxicity-based fractionation procedures (46,47,90) could prove particularly useful for identifying specific chemicals responsible for effects associated with complex mixtures (e.g., pulp and paper mill or municipal effluents, sediments).

Research Recommendations

The following compilation of research recommendations arises from the discussion above, with particular emphasis on the need to design initial investigations that will contribute to the ability to better define the extent and nature of current or potential EDC effects. This listing does not imply a prioritization; in many instances, work needs to proceed simultaneously on several fronts. It is important to note that many of the research issues identified, in particular the need to extrapolate between measurement and assessment endpoints and

among different measurement endpoints, are not restricted to EDCs as a class. However, due to the nature/mechanisms of action associated with effects manifested by endocrine disruptors, this research program may present a unique opportunity to simultaneously address these more generic ecological risk assessment issues.

1) Work needs to be done to better define linkages between potential measurement endpoints (usually made at the level of the individual) and assessment endpoints (which typically are at population or community levels). Similarly, linkages between measurement endpoints at different levels of biological organization need to be better defined. For example, induction of vitellogenin in male fish appears to be a very specific response to exposure to estrogen mimics, however, it is unclear what this means in terms of reproduction.

The basic challenge in this research area is to identify those endpoints that are indicative both of exposure to EDCs and predictive of their effects in populations. Part of this need includes research focused on better definition of normal conditions with respect to endocrine-regulated processes in commonly tested, or monitored, species relative to effects manifested at the population level. For example, the degree to

which circulating levels of sex steroids are altered before reproductive success is threatened needs to be defined.

2) There is a pressing need to identify the extent of the chemical universe about which there should be concern. This clearly cannot consist of testing all chemicals in long-term chronic assays with multiple species. Improved short-term *in vitro* and *in vivo* assays, as well as SAR models, are needed; however, all require further development and validation. The results of screening exercises using these types of tools then could be linked to key exposure data, such as production volume, persistence, etc., to help develop lists of chemicals of concern. Until comparisons of this type are accomplished it will be difficult, if not impossible, to coordinate a cohesive monitoring program focused upon defining the potential extent of the problem.

These types of screening tools should play significant roles not only in retrospective, but also in prospective assessments of the ecological risk of EDCs (e.g., for the premanufacture notification process under TSCA). A key consideration, of course, in developing these types of methods/models is the ability to link results obtained in suborganismal systems to adverse organismal-level effects.

3) Existing assays used in product testing or monitoring of environmental samples (e.g., effluents, sediments) should be evaluated and adapted, if necessary, to ensure exposure during key developmental windows, and evaluation of relevant (e.g., latent) endpoints for EDCs. Processes that should receive particular attention in this regard are reproduction, development and, to a lesser extent, immunocompetence. Where existing assays cannot be readily modified, new assays may have to be developed and validated.

4) The various measurement endpoints listed on Table 4, in particular those at the organismal level, need to be adapted to classes of organisms that have received little attention in terms of traditional toxicity test methods/approaches, such as amphibians, non-teleost fish, passerine birds and non-anthropod invertebrates. More specifically, development of a comparative endocrinology/toxicology knowledge base in potentially sensitive species is needed, and a better definition of baseline conditions for general processes and specific endocrine function is required. With advances in these areas, comparative endocrinology can better serve as a basis for assessing interspecies differences in susceptability to EDCs.

Research Strategies

The following specific research strategies were suggested over the course of the workshop to begin addressing the research recommendations listed above. It is anticipated that as organization-specific workplans are developed, the strategies will be further refined/modified.

1) Review and compile available data on endocrine function and endocrine cycles in species of concern (e.g, potential vulnerable species) to identify areas where additional research is needed.

2) Consolidate and review data from ongoing monitoring programs (e.g., EMAP, NAWQA, BEST) to identify trends that may be associated with effects of EDCs.

3) Modify existing monitoring programs to include information relevant to EDCs - e.g., measurement of relevant chemicals, information about sex ratios, endocrine parameters, etc.

4) Increase emphasis on research in endocrinology/toxicology to evaluate and improve current capabilities to identify potential EDCs and quantify organismal-level effects. Part of this would consist of a systematic evaluation of existing test protocols for various species. Further research in comparative endocrinology

and toxicology would also start to establish uncertainty “bounds” for interspecies extrapolation.

5) Conduct focused research projects at a few selected sites with known EDC problems. Examine multiple species at several levels of organization to establish linkages between endpoints measured in the laboratory at the suborganismal/individual level and changes in the field at higher levels of organization. Strategies and information developed from these projects could then be used to highlight specific knowledge gaps in ecological risk assessments and, in a related manner, be used to assess or predict impacts of EDCs in other areas, with a clearer understanding and appreciation of associated uncertainties.

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